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#72.

INVESTIGATIONS IN FISH CONTROL

72. Toxicity of Rotenone to Fish in Standardized Laboratory Tests

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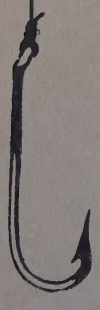
FISH & WILDLIFE SERVICE



United States Department of the Interior
Fish and Wildlife Service

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INVESTIGATIONS IN FISH CONTROL

72. Toxicity of Rotenone to Fish in Standardized Laboratory Tests

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Contents

	Page
Abstract	1
Materials and Methods	1
Results	2
Toxicity to various species of fish	2
Effects of temperature, water hardness, and pH on toxicity	2
Toxicity of Noxfish to green eggs of rainbow trout	2
Persistence of Noxfish in water	6
Detoxification of Noxfish	8
Toxicity of different formulations of rotenone	8
Toxicity in flow-through tests	8
Discussion	10
References	11

Toxicity of Rotenone to Fish in Standardized Laboratory Tests

by

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Abstract

Noxfish®, which contains 5% rotenone, was toxic to a variety of freshwater fish at concentrations ranging from 21.5 to 497 μg/l in 96-h laboratory exposures. Goldfish *(Carassius auratus)* and black bullheads *(Ictalurus melas)* were the most resistant species, and the Atlantic salmon *(Salmo salar)* was the most sensitive. Toxicity was influenced little by temperatures of 7 to 22 C, by water hardnesses of 10 to 300 mg/l, or by pH's of 6.5 to 9.5. In exposures of rainbow trout *(Salmo gairdneri)*, newly fertilized eggs were much more resistant than fingerlings. Noxfish detoxified in water solutions; the half-life of biological activity was 22 days at 12 C and 13 days at 17 C. Potassium permanganate was an excellent detoxifier; chlorine was less efficient. Noxfish was consistently more toxic in static tests than in flow-through tests.

Rotenone, a crystalline ketone found in several plants of the Leguminosae, has been used as a toxicant by fishery managers since the 1930's for removing undesired fish populations in lakes (Baker and Cordone 1969). Ideal conditions for the reclamation of static waters with rotenone include temperatures between 16 and 21 C, alkalinities between 150 and 200 mg/l (ppm), pH's of 8 or less, and surface areas of less than 8.1 ha (Spitler 1970). The piscicide has been used extensively under a wide variety of conditions and is relatively harmless to most nontarget organisms (Schnick 1974; Lennon et al. 1970). Twenty-nine formulations of rotenone from 18 companies had been registered for aquatic or agricultural use by 1974 by the Environmental Protection Agency. Because the registrations are old, data supporting the labels must be updated to conform to present requirements (Lennon 1967). A guide or protocol for evaluating the toxicity of candidate fishery chemicals for registration was published by Marking (1975).

The present study was designed to determine (1) the toxicity of rotenone to fish in standardized static and flow-through tests, (2) the toxicity of rotenone to newly fertilized trout eggs, (3) the residual toxicity of rotenone in water after selected periods of aging, (4) the efficiency of two compounds used to detoxify rotenone, and (5) the comparative toxicities of three rotenone formulations.

Materials and Methods

Three formulations of rotenone (furnished by S. B. Penick & Co.) were used in the tests described here: Noxfish®, an emulsifiable concentrate containing 5% rotenone; Pro-Noxfish®, a synergized emulsifiable concentrate containing 2.5% rotenone; and rotenone, a powder containing 33% rotenone. Except for tests in which the toxicities of the different formulations were compared or evaluated, Noxfish was used in all tests, and concentrations were based on the total amount of Noxfish formulation rather than on the amount of rotenone in the formulation. Stock solutions of the toxicants were prepared daily, and the portions needed to yield the desired concentrations were added to test chambers.

Static and flow-through test procedures followed those of Lennon and Walker (1964) and The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). In static tests, 10 fish were exposed to each concentration in glass jars containing 15 liters of oxygen-saturated test water prepared from deionized water (Marking 1969). Waters of four levels of hardness were used (total hardness as mg/l of $CaCO_3$ in parentheses): very soft (10), soft (44), hard (170), and very hard (300).

In separate tests to assess the effect of pH on toxicity, chemical buffers were added to control the pH (Marking 1975). Test temperatures were regulated

by immersing the test jars in constant temperature water baths. In flow-through toxicity tests, 20 fish were exposed to each concentration in a system similar to that described by Mount and Brungs (1967). Modifications included electronic microswitches to control cycling, pressure regulators, and an automatic pipette (Micromedic®) for injecting the toxicants into dilution water. Municipal water used for flow-through exposures was carbon filtered and had a total hardness of 320 mg/l and a pH of 7.5. Flow rates were maintained at about five chamber volumes per 24 h. Temperature was maintained with a water bath around the test chambers.

Fish weighing 1 to 1.5 g each were obtained from Federal hatcheries and maintained according to standardized procedures of the Fish Control Laboratory (Hunn et al. 1968). Scientific names for all species used are listed in Table 1. Fish were acclimated to the test conditions for 4 days before they were exposed to the toxicant. Mortalities were recorded at 1, 3, and 6 h on the first day of exposure and daily thereafter for the remainder of the test. Trout eggs were exposed to Noxfish in a manner similar to that used for fish, except that the static test vessel contained 2.5 liters of test solution. Details of methods for exposing eggs were those outlined by Olson and Marking (1973).

The methods of Litchfield and Wilcoxon (1949) were used in computation of the LC_{50}'s (concentrations producing 50% mortality) and 95% confidence intervals. Regressions were drawn and inspected for each set of data. All data reported fulfilled the chi-square test requirement for acceptability.

Deactivation indices for Noxfish were derived in soft water at temperatures of 12 and 17 C. Aged solutions of the toxicant were bioassayed to determine the biological activity remaining after selected time periods. The deactivation index was determined by dividing the LC_{50} of aged solutions by the LC_{50} of unaged solutions under corresponding test conditions (Marking 1972). The deactivation index was plotted against aging time on semilogarithmic coordinates to estimate the half-life of biological activity. Detoxification procedures with potassium permanganate ($KMnO_4$) and chlorine were those used by Marking and Bills (1975).

Results

Toxicity to Various Species of Fish

Noxfish was toxic to a wide variety of fish at concentrations ranging, in 96-h exposures, from 21.5 μg/l (or parts per billion) for Atlantic salmon to 497 μg/l for goldfish (Table 1). The 96-h LC_{50} was less than 100 μg/l for bowfins, all six salmonids, northern pike, carp, longnose and white suckers, smallmouth bass, yellow perch, and walleyes. The 96-h LC_{50} was greater than 100 μg/l for goldfish, fathead minnows, black bullheads, channel catfish, green sunfish, bluegills, and largemouth bass. Goldfish and black bullheads were most resistant—10 times as resistant as most other species. Generally, the resistant species required longer exposures than did the sensitive species, before they succumbed. None of the goldfish died in 24 h of exposure to high concentrations of Noxfish and none of the black bullheads in 6 h. On the other hand, most of the sensitive species died in 3-h exposures to much lower concentrations.

Effects of Temperature, Water Hardness, and pH on Toxicity

Noxfish was generally less toxic to rainbow trout, channel catfish, and bluegills at the lower than at the higher temperatures in 3- and 6-h exposures (Tables 2, 3, and 4). After 96 h this trend remained but the differences in toxicity associated with most 5° differences in temperature were insignificant. The difference was significant for rainbow trout, however, at 7 and 12 C. Trout were consistently more sensitive than channel catfish or bluegills.

Water hardness had no effect on toxicity of Noxfish (Tables 2, 3, and 4), with one exception; in 96-h exposures the LC_{50}'s for channel catfish were 277 μg/l in soft water and 328 μg/l in very soft water (Table 3). Water at each hardness contained 384 mg/l of sodium bicarbonate to equalize the pH at about 8.0, and that quantity of bicarbonate in the soft water presumably resulted in slightly decreased toxicity to rainbow trout and channel catfish.

The toxicity of Noxfish was not influenced by differences in pH within the range of 6.5 to 9.5 (Tables 2, 3, and 4). Noxfish appeared to be more toxic to rainbow trout at pH 9.5 than at lower pH's, but the increased sensitivity might have been due to an inability of the trout to acclimate fully to the high pH. Bluegills responded uniformly at the three different pH's in soft water at 12 C; the 96-h LC_{50}'s ranged only from 122 to 138 μg/l.

Toxicity of Noxfish to Green Eggs of Rainbow Trout

Newly fertilized eggs of rainbow trout were 47 to 106 times more resistant than fingerlings to Noxfish; the 96-h LC_{50} ranged from 5.60 mg/l in very soft water to 2.50 mg/l in very hard water (Table 5).

Table 1. *Toxicity of Noxfish® to fish in standardized laboratory tests at 12 C.*

Species	LC_{50} and 95% confidence interval (μg/l) at			
	3 h	6 h	24 h	96 h
Bowfin *Amia calva*	141 114-174	106 82.5-136	57.5 50.4-65.5	30.0 23.7-38.0
Coho salmon *Oncorhynchus kisutch*	358 —	152 105-219	71.6 63.1-81.3	62.0 54.8-70.2
Chinook salmon *O. tshawytscha*	212 171-262	156 137-177	49.0 44.3-54.2	36.9 33.9-40.2
Rainbow trout *Salmo gairdneri*	175 160-191	86.9 —	68.9 56.2-84.4	46.0 32.6-64.9
Atlantic salmon *S. salar*	61.5 53.4-70.8	40.0 33.6-70.8	35.0 29.7-41.2	21.5 15.5-29.8
Brook trout *Salvelinus fontinalis*	141 124-160	79.7 69.2-91.8	47.0 42.2-52.3	44.3 41.1-47.7
Lake trout *S. namaycush*	50.0 38.6-64.7	28.3 21.0-38.0	26.9 19.8-36.5	26.9 19.8-36.5
Northern pike *Esox lucius*	181 160-204	58.2 52.5-64.5	44.9 31.4-64.3	33.0 26.6-41.0
Goldfish *Carassius auratus*	—	—	—	497 412-600
Carp *Cyprinus carpio*	—	270 254-287	84.0 74.7-94.4	50.0 41.1-60.8
Fathead minnow *Pimephales promelas*	—	1,190 917-1,453	400 291-549	142 115-176
Longnose sucker *Catostomus catostomus*	388 332-454	218 141-337	67.2 59.3-76.1	57.0 51.9-62.6
White sucker *C. commersoni*	630 452-878	238 186-304	71.9 64.0-80.8	68.0 54.0-85.6
Black bullhead *Ictalurus melas*	—	—	665 516-856	389 298-507
Channel catfish *I. punctatus*	1,410 1,139-1,745	840 717-984	400 234-684	164 138-196
Green sunfish *Lepomis cyanellus*	389 332-456	332 249-443	218 197-241	141 114-174
Bluegill *L. macrochirus*	424 335-537	336 245-461	149 124-178	141 133-149
Smallmouth bass *Micropterus dolomieui*	277 219-350	165 —	93.2 85.1-102	79.0 70.7-88.2
Largemouth bass *M. salmoides*	514 449-588	360 305-425	200 131-305	142 115-176
Yellow perch *Perca flavescens*	150 126-179	134 120-149	92.0 80.1-106	70.0 59.8-82.0
Walleye *Stizostedion vitreum vitreum*	135 103-176	52.4 46.8-58.7	16.5 15.2-17.9	—

Table 2. *Toxicity of Noxfish® to rainbow trout in water of different temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (μg/l) at			
			3 h	6 h	24 h	96 h
7	Soft	7.5	>400	276 237-322	158 134-186	70.0 62.5-78.4
12	Soft	7.5	175 160-191	86.9 —	68.9 56.2-84.4	46.0 32.6-64.9
17	Soft	7.5	73.0 59.8-89.1	73.0 59.8-89.1	43.4 30.9-60.9	43.4 30.9-60.9
12	Very soft	8.0	122 108-138	61.9 54.9-70.2	54.4 45.9-64.4	54.4 45.9-64.4
12	Soft	8.0	90.0 81.1-99.9	62.0 51.6-74.5	56.5 48.2-66.3	56.5 48.2-66.3
12	Hard	8.0	112 95.1-132	81.9 64.5-104	55.1 43.9-69.	55.1 43.9-69.1
12	Very hard	8.0	113 92.7-138	66.9 55.0-81.4	53.0 44.3-63.4	53.0 44.3-63.4
12	Soft	6.5	169 151-189	129 107-155	78.5 69.6-88.6	69.5 63.7-75.9
12	Soft	8.5	133 108-163	98.0 87.4-110	80.0 70.3-91.0	62.1 51.7-74.5
12	Soft	9.5	124 106-145	75.0 63.2-89.0	54.0 45.9-63.6	35.5 28.7-43.9

Table 3. *Toxicity of Noxfish® to channel catfish at selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (μg/l) at 3 h	6 h	24 h	96 h
12	Soft	7.5	1,720 1,381-2,141	1,000 784-1,276	539 377-770	200 164-244
17	Soft	7.5	1,410 1,139-1,745	840 717-984	400 234-684	164 138-196
22	Soft	7.5	739 672-813	449 352-572	164 137-196	164 137-196
12	Very soft	8.0	1,420 1,080-1,868	640 464-883	476 346-655	328 290-370
12	Soft	8.0	1,640 1,341-2,005	890 677-1,171	450 341-593	237 199-282
12	Hard	8.0	1,220 1,023-1,454	942 756-1,173	400 312-513	308 237-400
12	Very hard	8.0	1,160 996-1,351	1,000 782-1,279	359 282-457	318 240-421
12	Soft	6.5	1,530 1,175-1,991	865 692-1081	500 326-767	200 158-254
12	Soft	8.5	899 689-1,173	735 608-889	565 454-704	309 237-403
12	Soft	9.5	629 548-721	625 546-715	550 444-681	248 184-335

Table 4. *Toxicity of Noxfish® to bluegills at selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (μg/l) at 3 h	6 h	24 h	96 h
12	Soft	7.5	450 353-573	270 217-335	141 114-174	141 114-174
17	Soft	7.5	424 335-537	336 245-461	149 124-178	141 133-149
22	Soft	7.5	268 240-300	227 194-266	140 107-183	132 122-143
12	Very soft	8.0	334 194-575	219 183-262	142 129-157	132 118-147
12	Soft	8.0	450 317-639	319 241-422	152 135-172	137 123-153
12	Hard	8.0	300 196-460	284 200-403	146 131-162	132 118-147
12	Very hard	8.0	295 211-413	194 148-255	138 125-152	132 121-144
12	Soft	6.5	291 207-409	228 194-267	150 124-181	138 110-173
12	Soft	8.5	255 204-319	192 170-217	122 108-138	122 108-128
12	Soft	9.5	196 162-237	152 134-173	122 108-138	122 108-138

Although the difference in toxicity was not significant at each hardness increment, the difference was significant in very soft as compared to hard or very hard water.

Table 5. *Toxicity of Noxfish® to newly fertilized eggs of rainbow trout in reconstituted water of different hardnesses at 12 C.*

Water hardness	96-h LC_{50} (mg/l) and 96% confidence interval
Very soft	5.60 3.55-8.83
Soft	4.42 3.28-5.96
Hard	3.20 2.31-4.43
Very hard	2.50 2.16-2.90

Persistence of Noxfish in Water

The toxicity to bluegills of Noxfish solutions aged for 1, 2, and 3 weeks decreased through each week of aging. At 12 C the 96-h LC_{50}'s were 133 μg/l in freshly prepared solutions and 254 μg/l in solutions aged for 3 weeks (Table 6). The toxicity decreased

Table 6. *Toxicity (96-h LC_{50}'s and 95% confidence intervals in μg/l) to bluegills of fresh and aged solutions of Noxfish® in soft water (deactivation indices shown in parentheses).*

Temp (°C)	Aging time (weeks) 0	1	2	3	Half-life (days)
12	133 117-151 (1.00)	—	213 193-236 (1.60)	254 215-300 (1.91)	22
17	90.0 64.1-126 (1.00)	117 92.3-148 (1.30)	190 159-228 (2.11)	288 247-336 (3.20)	13

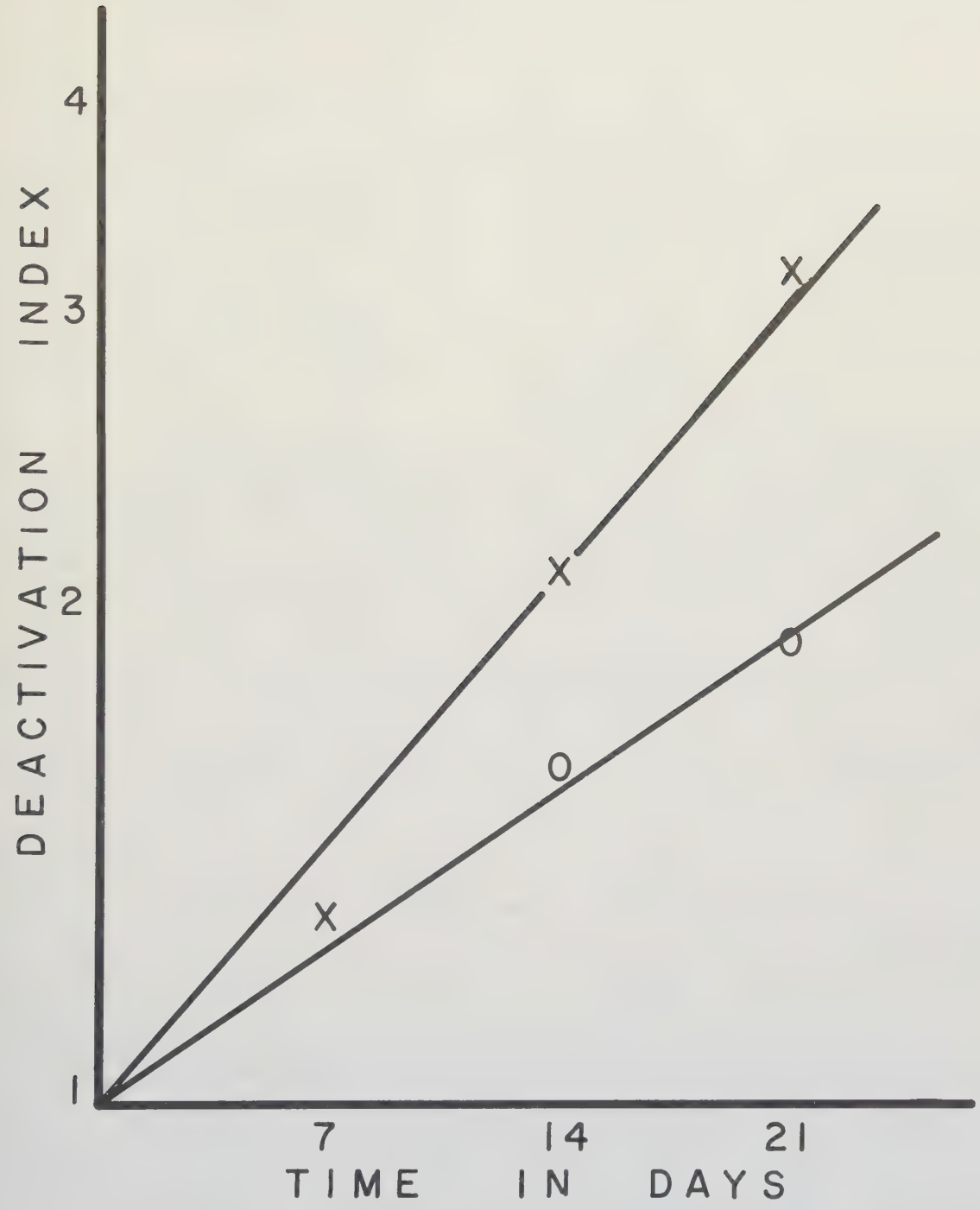

Fig. 1. Detoxification rate for Noxfish® in soft water at 12 C [O] and 17 C [X].

more rapidly at 17 C than at 12 C; the half-lives were 13 and 22 days, respectively (Fig. 1).

Detoxification of Noxfish

Static tests with green sunfish showed that potassium permanganate immediately detoxified Noxfish, as indicated by the marked loss of activity in unaged solutions of Noxfish and $KMnO_4$ (Table 7). For instance, the 96-h LC_{50} at pH 7.5 was 0.241 mg/l for Noxfish alone and 1.71 mg/l for Noxfish plus 1.0 mg/l of $KMnO_4$. The solutions of Noxfish and $KMnO_4$ that were aged for 50 min before the fish were added had lost some additional activity (96-h LC_{50}, 3.09). The immediate detoxification in unaged solutions was probably a reflection of the effective exposure time, i.e., the time required for Noxfish to produce a lethal effect. The $KMnO_4$ detoxified Noxfish in water at all pH's tested.

Chlorine was far less effective than $KMnO_4$ for detoxifying Noxfish in laboratory tests. There was little immediate detoxification in unaged solutions and little detoxification during 6 h of aging. For instance, at pH 7.5 the 96-h LC_{50} was 0.293 mg/l for Noxfish alone and 0.429 mg/l for Noxfish plus 0.5 mg/l of chlorine (Table 8). In a 6-h aging period, Noxfish was detoxified more efficiently at pH 9.5 than at pH's 7.5 and 8.5.

Toxicity of Different Formulations of Rotenone

Pro-Noxfish, a synergized formulation containing 2.5% rotenone, was more toxic to rainbow trout than the Noxfish formulation or powdered rotenone when concentrations were calculated on the basis of rotenone content. The comparative 96-h LC_{50}'s ($\mu g/l$) were as follows: Pro-Noxfish, 1.02; Noxfish, 3.05; and powdered rotenone, 4.20 (Table 9). There was no significant difference in toxicity between Noxfish and rotenone powder formulations.

Toxicity in Flow-through Tests

In 4-day flow-through exposures, Noxfish was more toxic to chinook salmon and yellow perch than to carp or white suckers (Table 10). Toxicity did not increase with exposure time after 4 days, except for carp (in which toxicity increased through 20 days). Noxfish

Table 7. *Toxicity and detoxification of Noxfish® in static tests with green sunfish in water containing 1.0 mg/l of $KMnO_4$ at 12 C.*

Compound and (for compounds combined) interaction time (min)[a]	96-h LC_{50} and 95% confidence interval (mg/l) at			
	pH 6.5	pH 7.5	pH 8.5	pH 9.5
$KMnO_4$	3.47 3.12–3.87	3.03 2.69–3.41	1.41 1.14–1.74	3.08 2.32–4.08
Noxfish	0.184 0.161–0.211	0.241 0.203–0.287	0.158 0.114–0.219	0.378 0.317–0.451
Noxfish + $KMnO_4$				
0	1.32 1.04–1.68	1.71 1.49–1.96	2.10 1.91–2.31	1.55 1.16–2.08
10	1.17 0.948–1.44	1.41 1.14–1.74	1.81 1.54–2.13	1.36 1.07–1.70
20	1.41 1.14–1.74	2.89 2.28–3.36	2.38 2.24–2.53	1.64 1.38–1.94
30	1.82 1.54–2.15	2.89 2.28–3.66	3.10 2.69–3.58	1.91 1.59–2.29
40	2.00 1.64–2.44	2.28 1.82–2.85	3.09 2.88–3.32	1.41 1.14–1.74
50	1.93 1.61–2.31	3.09 2.74–3.49	3.59 3.19–4.03	1.81 1.53–2.13

[a] Length of time Noxfish and $KMnO_4$ were added before fish were introduced.

Table 8. *Toxicity and detoxification of Noxfish® in static tests with green sunfish in water containing 0.5 mg/l of chlorine at 12 C.*

Compound and (for compounds combined) interaction time (h)[a]	96-h LC_{50} and 95% confidence interval (mg/l) at		
	pH 7.5	pH 8.5	pH 9.5
Chlorine	0.840 0.703–1.00	0.820 0.588–1.14	0.709 0.567–0.887
Noxfish	0.293 0.264–0.325	0.338 0.304–0.376	0.329 0.294–0.368
Noxfish + chlorine			
0	0.429 0.359–0.512	0.300 0.242–0.372	0.488 0.426–0.559
0.5	0.348 0.299–0.405	0.492 0.427–0.567	— —
1.0	0.483 0.406–0.575	0.380 0.318–0.455	0.900 0.677–1.20
2.0	0.412 0.337–0.504	0.400 0.339–0.473	0.689 0.584–0.813
4.0	0.494 0.438–0.557	0.489 0.432–0.554	0.770 0.689–0.862
6.0	0.494 0.438–0.557	0.454 0.390–0.529	1.37 0.988–1.90

[a] Length of time Noxfish and chlorine were added before fish were introduced.

Table 9. *Toxicity of three formulations of rotenone to rainbow trout in soft water at 12 C.*

Preparation	% active rotenone	LC_{50} and 95% confidence interval (μg/l) at				
		1 h	3 h	6 h	24 h	96 h
Pro-Noxfish®	2.5	13.0 8.15–20.7	4.53 3.28–5.65	2.98 2.43–3.65	1.82 1.60–2.08	1.02 0.917–1.15
Noxfish®	5.0	25.5 16.1–40.5	8.70 6.90–11.0	5.50 4.87–6.20	3.25 2.78–3.81	3.05 2.85–3.27
Powdered rotenone	33.0	16.5 14.3–19.1	8.09 6.37–10.3	6.60 5.41–8.02	3.82 3.30–4.46	3.20 2.09–3.70

Table 10. *Toxicity of Noxfish® to four species of fish in flow-through toxicity tests at 12 C.*

Species	LC_{50} and 95% confidence interval (μg/l) at				
	1 day	4 days	10 days	20 days	30 days
Chinook salmon	112 97.7-128	71.0 55.3-99.1	62.0 52.1-73.7	59.0 49.5-70.3	—
Carp	—	142 122-165	96.0 78.0-118	67.0 57.4-78.3	68.0 57.7-80.1
White sucker	—	144 122-170	129 118-141	112 95.5-131	112 95.5-131
Yellow perch	160 121-211	60.0 53.3-67.6	50.0 36.8-68.0	46.0 32.7-64.8	

was consistently and significantly more toxic to all four species in static than in flow-through tests (Table 11).

Discussion

The literature on toxicity of rotenone to fish suggests that concentrations used in fishery management are generally higher than those known to be lethal in laboratory tests; that toxicity depends on temperature, water hardness, pH, and physical characteristics; and that many different application rates may be effective for the same target species of fish (Schnick 1974; Meyer 1966).

Since laboratory procedures are usually more standardized than field procedures, laboratory data are expected to be more consistent than field data. Although the LC_{50}'s of less than 0.2 mg/l for Noxfish against rainbow trout, channel catfish, and bluegills reported by Bridges and Cope (1965) were similar to ours, applications of at least 1 mg/l have been repeatedly recommended for eliminating these species. Spitler (1970) reported that 1.6 mg/l of Noxfish was not effective and that as much as 5 mg/l was sometimes needed. The difference in laboratory and field data is due to several factors. Laboratory data generally indicate concentrations that produce 50% mortality (LC_{50}), whereas field concentrations are based on eliminating 100% of the target fish. Organisms, particulate matter, and sunlight contribute to the tendency toward faster detoxification of chemicals in natural waters than in the laboratory. Furthermore, because uniform concentrations are much more difficult to obtain in the field, additional amounts of toxicants are generally applied to ensure a lethal concentration throughout a body of water.

Table 11. *Comparison of acute toxicities of Noxfish® to four species of fish in 96-h flow-through and static tests in carbon filtered municipal water at 12 C.*

Species	LC_{50} and 95% confidence interval (μg/l)	
	Static	Flow-through
Chinook salmon	34.7 26.9-44.7	71.0 55.3-99.1
Carp	19.0 12.1-29.9	142 122-165
White sucker	17.9 12.8-25.1	144 122-170
Yellow perch	30.0 23.6-38.2	60.0 53.3-67.6

Although some of the reports are conflicting, rotenone is generally more effective at high than at low temperatures (Gersdorff 1943; Almquist 1959; Ball 1948; Hooper 1955), in acid than in alkaline waters (Leonard 1939; Foye 1964), and in soft than in hard water (Foye 1964). In many of these studies, however, efficacy was based on survival time of the fish rather than on the concentration of the toxicant. Our laboratory data show only slight changes in the toxicity of rotenone at different temperatures, hardnesses, or pH's. Consequently, concentrations used in the field should be based on the results of on-site toxicity tests (Burress 1975) rather than on extrapolations of laboratory or field data.

Most studies—in laboratory or field—have shown that goldfish and black bullheads are the species most resistant to rotenone. Individual fish of a species may be exceptionally resistant (Meyer 1966)—an observation that may explain some incomplete fish kills and the need to apply a concentration

greater than that indicated in laboratory tests.

The detoxifiers $KMnO_4$ and chlorine were toxic to fish at concentrations only slightly greater than those needed to detoxify rotenone. For example, against green sunfish in water at pH 8.5, the 96-h LC_{50} for $KMnO_4$ was 1.41 mg/l and that for chlorine was 0.82 mg/l. These results support Engstrom-Heg and Loeb (1968) and Engstrom-Heg (1972), who cautioned that high concentrations of $KMnO_4$ may become toxic and may have to be reduced with sodium thiosulfate or other agents. Therefore, detoxifiers should be used only when necessary and in only the quantities needed.

References

Almquist, E. 1959. Observations on the effect of rotenone emulsives on fish food organisms. Inst. Freshwater Res. Drottningholm Rep. 40:146–160.

Baker, P. H., and A. J. Cordone. 1969. Distribution, size composition, and relative abundance of the Piute sculpin, *Cottus beldingii* Eigenmann and Eigenmann, in Lake Tahoe. Calif. Fish Game 55(4):285–297.

Ball, R. C. 1948. A summary of experiments in Michigan lakes on the elimination of fish populations with rotenone, 1934–1942. Trans. Am. Fish. Soc. 75(1945):139–146.

Bridges, W. R., and O. B. Cope. 1965. The relative toxicities of similar formulations of pyrethrum and rotenone to fish and immature stoneflies. Pyrethrum Post 8(1):3–5.

Burress, R. M. 1975. Development and evaluation of on-site toxicity test procedures for fishery investigations. U.S. Fish Wildl. Serv. Invest. Fish Control 68. 8 pp.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecol. Res. Ser. EPA [Environ. Prot. Agency]-660/3-75-009. 61 pp.

Engstrom-Heg, R. 1972. Kinetics of rotenone-potassium permanganate reactions as applied to the protection of trout streams. N.Y. Fish Game J. 19(1):47–58.

Engstrom-Heg, R., and H. A. Loeb. 1968. Chemical detoxification of rotenone: a simple case of oxidation? N.Y. Conserv. Dep., Bur. Fish, N.Y. Fish News 27:3–7.

Foye, R. E. 1964. Chemical reclamation of forty-eight ponds in Maine. Prog. Fish-Cult. 26(4):181–185.

Gersdorff, W. A. 1943. Effect of change of temperature on relative toxicity of rotenone and phenol. J. Agric. Res. 67(2):65–80.

Hooper, F. F. 1955. Eradication of fish by chemical treatment. Mich. Dep. Conserv., Fish Div. Pam. No. 19. 6 pp.

Hunn, J. B., R. A. Schoettger, and E. W. Whealdon. 1968. Observations on the handling and maintenance of bioassay fish. Prog. Fish-Cult. 30(3):164–167.

Lennon, R. E. 1967. Clearance and registration of chemical tools for fisheries. Prog. Fish-Cult. 29(4):187–193.

Lennon, R. E., and C. R. Walker. 1964. Laboratories and methods for screening fish-control chemicals. U.S. Fish Wildl. Serv. Invest. Fish Control 1 (Circ. 185). 15 pp.

Lennon, R. E., J. B. Hunn, R. A. Schnick, and R. M. Burress. 1970. Reclamation of ponds, lakes, and streams with fish toxicants: a review. FAO Fish. Tech. Pap. No. 100. 99 pp.

Leonard, J. W. 1939. Notes on the use of derris as a fish poison. Trans. Am. Fish. Soc. 68(1938):269–279.

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96(2):99–113.

Marking, L. L. 1969. Toxicological assays with fish. Bull. Wildl. Dis. Assoc. 5:291–294.

Marking, L. L. 1972. Methods of estimating the half-life of biological activity of toxic chemicals in water. U.S. Fish Wildl. Serv. Invest. Fish Control 46. 9 pp.

Marking, L. L. 1975. Toxicological protocol for the development of piscicides. Pages 26–31 *in* P. H. Eschmeyer, ed. Rehabilitation of fish populations with toxicants: a symposium. Am. Fish. Soc., North Central Div., Spec. Publ. No. 4.

Marking, L. L., and T. D. Bills. 1975. Toxicity of potassium permanganate to fish and its effectiveness for detoxifying antimycin. Trans. Am. Fish. Soc. 104(3):579–583.

Meyer, F. A. 1966. Chemical control of undesirable fishes. Pages 498–510 *in* A. Calhoun, ed. Inland fisheries management. Calif. Dep. Fish Game, Sacramento.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. Water Res. 1:21–29.

Olson, L. E., and L. L. Marking. 1973. Toxicity of TFM (lampricide) to six early life stages of rainbow trout *(Salmo gairdneri)*. J. Fish. Res. Board Can. 30:1047–1052.

Schnick, R. A. 1974. A review of the literature on use of rotenone in fisheries. U.S. Fish Wildl. Serv. Lit. Rev. 74–15. NTIS [Natl. Tech. Inf. Serv.] No. PB-235 454/AS. 130 pp.

Spitler, R. J. 1970. An analysis of rotenone treatments for estimation of fish populations in southern Michigan lakes, 1957–1967. Mich. Acad. 3(1):77–82.

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